

SEQUENCE LISTING

1528960
DT01 Rec'd PCT/PTO 22 MAR 2005

<110> DSM IP ASSETS B.V.

<120> Enone reductase gene

<130> NDR5231

<140> PCT/EP03/10473

<141> 2003-09-19

<150> EP 02021098.5

<151> 2002-09-23

<160> 32

<170> PatentIn version 3.2

<210> 1

<211> 1212

<212> DNA

<213> Candida kefyr

<220>

<221> CDS

<222> (1)..(1212)

<400> 1

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Met	Ser	Tyr	Met	Asn	Phe	Asp	Pro	Lys	Pro	Leu	Gly	Asp	Thr	Asn	Ile	
1				5					10					15		

ttc	aag	cca	atc	aag	atc	ggt	aac	aat	gag	cta	aaa	cac	aga	gta	gtc	96
Phe	Lys	Pro	Ile	Lys	Ile	Gly	Asn	Asn	Glu	Leu	Lys	His	Arg	Val	Val	
			20					25					30			

atg	cca	gca	ttg	act	aga	atg	aga	gcc	att	gca	cca	gga	aac	atc	cca	144
Met	Pro	Ala	Leu	Thr	Arg	Met	Arg	Ala	Ile	Ala	Pro	Gly	Asn	Ile	Pro	
		35					40					45				

aac	act	gaa	tgg	gcc	gag	gaa	tac	tac	aga	caa	cgt	tct	caa	tac	cct	192
Asn	Thr	Glu	Trp	Ala	Glu	Glu	Tyr	Tyr	Arg	Gln	Arg	Ser	Gln	Tyr	Pro	
	50				55						60					

ggt	acc	ctt	att	atc	acg	gaa	ggt	act	ttc	cct	tct	gcg	caa	tca	ggt	240
Gly	Thr	Leu	Ile	Ile	Thr	Glu	Gly	Thr	Phe	Pro	Ser	Ala	Gln	Ser	Gly	
65					70					75					80	

ggt	tac	cca	aat	gtg	cca	ggt	atc	tgg	tcc	aaa	gag	caa	ttg	gct	gaa	288
Gly	Tyr	Pro	Asn	Val	Pro	Gly	Ile	Trp	Ser	Lys	Glu	Gln	Leu	Ala	Glu	
				85				90						95		

tgg	aaa	aag	atc	ttc	aat	gca	atc	cat	gag	aac	aaa	tcg	ttc	gtg	tgg	336
Trp	Lys	Lys	Ile	Phe	Asn	Ala	Ile	His	Glu	Asn	Lys	Ser	Phe	Val	Trp	
			100					105					110			

gtg	caa	ttg	tgg	gtt	cta	ggt	aga	caa	gca	tgg	cca	gaa	gtg	ttg	aag	384
Val	Gln	Leu	Trp	Val	Leu	Gly	Arg	Gln	Ala	Trp	Pro	Glu	Val	Leu	Lys	
		115					120					125				

aag	gaa	ggt	ttg	cgt	tac	gat	agt	gct	acc	gat	gac	ttg	tac	atg	ggt	432
Lys	Glu	Gly	Leu	Arg	Tyr	Asp	Ser	Ala	Thr	Asp	Asp	Leu	Tyr	Met	Gly	
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Lys Lys Tyr

<210> 2
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<212> PRT
<213> Candida kefyr

<400> 2

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Phe Lys Pro Ile Lys Ile Gly Asn Asn Glu Leu Lys His Arg Val Val
20 25 30

Met Pro Ala Leu Thr Arg Met Arg Ala Ile Ala Pro Gly Asn Ile Pro
35 40 45

Asn Thr Glu Trp Ala Glu Glu Tyr Tyr Arg Gln Arg Ser Gln Tyr Pro
50 55 60

Gly Thr Leu Ile Ile Thr Glu Gly Thr Phe Pro Ser Ala Gln Ser Gly
65 70 75 80

Gly Tyr Pro Asn Val Pro Gly Ile Trp Ser Lys Glu Gln Leu Ala Glu
85 90 95

Trp Lys Lys Ile Phe Asn Ala Ile His Glu Asn Lys Ser Phe Val Trp
100 105 110

Val Gln Leu Trp Val Leu Gly Arg Gln Ala Trp Pro Glu Val Leu Lys
115 120 125

Lys Glu Gly Leu Arg Tyr Asp Ser Ala Thr Asp Asp Leu Tyr Met Gly
130 135 140

Glu Glu Glu Lys Glu Arg Ala Leu Lys Ala Asn Asn Pro Gln His Gly
145 150 155 160

Ile Thr Lys Glu Glu Ile Lys Gln Tyr Ile Lys Glu Tyr Val Asp Ala
165 170 175

Ala Lys Lys Ala Ile Asp Ala Gly Ala Asp Gly Val Gln Ile His Ser
180 185 190

Ala Asn Gly Tyr Leu Leu Asn Gln Phe Leu Asp Pro Ile Ser Asn Asn
195 200 205

Arg Thr Asp Glu Tyr Gly Gly Ser Ile Glu Asn Arg Ala Arg Phe Thr
210 215 220

Leu Glu Val Val Asp Ala Val Val Asp Ala Val Gly Ala Glu Arg Thr
225 230 235 240

Ser Ile Arg Phe Ser Pro Tyr Gly Thr Phe Gly Thr Met Ser Gly Gly
245 250 255

Glu Asn Pro Gly Ile Val Ala Gln Tyr Ala Tyr Val Ile Gly Glu Leu
260 265 270

Glu Lys Arg Ala Arg Ala Gly Lys Arg Leu Ala Phe Ile Asp Leu Val
275 280 285

Glu Pro Arg Val Thr Asp Pro Phe Leu Pro Glu Phe Glu Lys Trp Phe
290 295 300

Lys Glu Gly Thr Asn Glu Phe Ile Tyr Ser Ile Trp Lys Gly Pro Val
305 310 315 320

Leu Arg Val Gly Asn Tyr Ala Leu Asp Pro Asp Gln Ala Thr Leu Asp
325 330 335

Ser Lys Lys Pro Asn Thr Leu Ile Gly Tyr Gly Arg Ser Phe Ile Ala
340 345 350

Asn Pro Asp Leu Val Tyr Arg Leu Glu Lys Gly Leu Pro Leu Asn Lys
355 360 365

Tyr Asp Arg Asn Thr Phe Tyr Thr Phe Thr Lys Glu Gly Tyr Thr Asp
370 375 380

Tyr Pro Ser Tyr Glu Glu Ser Val Ala Lys Gly Tyr Lys Lys Glu Glu
385 390 395 400

Lys Lys Tyr

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<213> Candida kefyr

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1 5 10

<210> 4
<211> 50
<212> PRT
<213> Candida kefyr

<400> 4

His Arg Val Val Met Pro Ala Leu Thr Arg Met Arg Ala Ile Ala Pro
1 5 10 15

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Ser Gln Tyr Pro Gly Thr Leu Ile Ile Thr Glu Gly Thr Phe Pro Ser
35 40 45

Val Gln
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<210> 5

<211> 8

<212> PRT

<213> Candida kefyr

<400> 5

Glu Gln Leu Ala Glu Trp Lys Lys
1 5

<210> 6

<211> 9

<212> PRT

<213> Candida kefyr

<400> 6

Ile Phe Asn Ala Ile His Glu Asn Lys
1 5

<210> 7

<211> 18

<212> PRT

<213> Candida kefyr

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<221> MISC_FEATURE

<222> (15)..(15)

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<222> (15)..(15)

<223> Xaa is unknown

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Glu val

<210> 8
<211> 19
<212> PRT
<213> Candida kefyr

<400> 8

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1 5 10 15

Glu Glu Lys

<210> 9
<211> 10
<212> PRT
<213> Candida kefyr

<400> 9

Ala Asn Asn Pro Gln His Gly Ile Thr Lys
1 5 10

<210> 10
<211> 7
<212> PRT
<213> Candida kefyr

<400> 10

Glu Tyr Val Asp Ala Ala Lys
1 5

<210> 11
<211> 50
<212> PRT
<213> Candida kefyr

<220>
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<222> (43)..(43)
<223> Xaa is unknown

<220>
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<223> Xaa is unknown

<220>
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<222> (47)..(48)
<223> Xaa is unknown

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Tyr Leu Leu Asn Gln Phe Leu Asp Pro Ile Ser Asn Asn Arg Thr Asp
20 25 30

Glu Tyr Gly Gly Ser Ile Ile Asn Arg Ala Xaa Phe Xaa Leu Xaa Xaa
35 40 45

Val Asp
50

<210> 12
<211> 22
<212> PRT
<213> Candida kefyr

<400> 12

Arg Leu Ala Phe Ile Asp Leu Val Glu Pro Arg Val Thr Asp Pro Phe
1 5 10 15

Leu Pro Glu Phe Glu Lys
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<210> 13
<211> 12
<212> PRT
<213> Candida kefyr

<400> 13

Glu Gly Thr Asn Glu Phe Ile Tyr Ser Ile Trp Lys
1 5 10

<210> 14
<211> 21
<212> PRT
<213> Candida kefyr

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Gly Pro Val Leu Arg Val Gly Asn Tyr Ala Leu Asp Pro Asp Gln Ala
1 5 10 15

Thr Leu Asp Ser Lys
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<210> 15
<211> 24
<212> PRT
<213> Candida kefyr

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1 5 10 15

Asp Leu Val Tyr Arg Leu Glu Lys

<210> 16
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 <213> Candida kefyr

<400> 16

Gly Leu Pro Leu Asn Lys
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<210> 17
 <211> 11
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 <213> Candida kefyr

<400> 17

Tyr Asp Arg Asn Thr Phe Tyr Thr Phe Thr Lys
 1 5 10

<210> 18
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 <213> Candida kefyr

<400> 18

Glu Gly Tyr Thr Asp Tyr Pro Ser Tyr Glu Glu Ser Val Ala Lys
 1 5 10 15

<210> 19
 <211> 9
 <212> PRT
 <213> Candida kefyr

<400> 19

Gly Asp Thr Asn Ile Phe Lys Pro Ile
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<210> 20
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 <213> Artificial Sequence

<220>
 <223> reverse sequence of a partial amino acid sequence of enone reductase

<400> 20

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<210> 21
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 <212> DNA

<213> Candida kefyr

<220>

<221> CDS

<222> (1)..(1056)

<400> 21

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gga Gly	aac Asn	atc Ile	cca Pro 20	aac Asn	act Thr	gaa Glu	tgg Trp	gcc Ala 25	gag Glu	gaa Glu	tac Tyr	tac Tyr	aga Arg 30	caa Gln	cgt Arg	96
tct Ser	caa Gln	tac Tyr 35	cct Pro	ggg Gly	acc Thr	ctt Leu	att Ile 40	atc Ile	acg Thr	gaa Glu	ggg Gly	act Thr 45	ttc Phe	cct Pro	tct Ser	144
gcg Ala	caa Gln 50	tca Ser	ggg Gly	ggg Gly	tac Tyr	cca Pro 55	aat Asn	gtg Val	cca Pro	ggg Gly	atc Ile 60	tgg Trp	tcc Ser	aaa Lys	gag Glu	192
caa Gln 65	ttg Leu	gct Ala	gaa Glu	tgg Trp	aaa Lys 70	aag Lys	atc Ile	ttc Phe	aat Asn	gca Ala 75	atc Ile	cat His	gag Glu	aac Asn	aaa Lys 80	240
tcg Ser	ttc Phe	gtg Val	tgg Trp	gtg Val 85	caa Gln	ttg Leu	tgg Trp	gtt Val	cta Leu 90	ggg Gly	aga Arg	caa Gln	gca Ala	tgg Trp 95	cca Pro	288
gaa Glu	gtg Val	ttg Leu	aag Lys 100	aag Lys	gaa Glu	ggg Gly	ttg Leu	cgt Arg 105	tac Tyr	gat Asp	agt Ser	gct Ala	acc Thr 110	gat Asp	gac Asp	336
ttg Leu	tac Tyr	atg Met 115	ggg Gly	gaa Glu	gaa Glu	gaa Glu	aaa Lys 120	gag Glu	cgt Arg	gcc Ala	tta Leu	aag Lys 125	gct Ala	aac Asn	aac Asn	384
cca Pro	cag Gln 130	cac His	ggg Gly	atc Ile	acc Thr	aag Lys 135	gaa Glu	gaa Glu	atc Ile	aag Lys	cag Gln 140	tac Tyr	atc Ile	aag Lys	gag Glu	432
tac Tyr 145	gtg Val	gat Asp	gct Ala	gcc Ala	aag Lys 150	aaa Lys	gcc Ala	atc Ile	gat Asp	gca Ala 155	ggg Gly	gca Ala	gac Asp	ggg Gly	gtg Val 160	480
caa Gln	atc Ile	cat His	tct Ser	gcc Ala 165	aac Asn	ggg Gly	tac Tyr	ttg Leu	ttg Leu 170	aac Asn	cag Gln	ttt Phe	ttg Leu	gac Asp 175	cct Pro	528
att Ile	tct Ser	aac Asn 180	aac Asn	aga Arg	acc Thr	gac Asp	gag Glu	tac Tyr 185	ggg Gly	gga Gly	tcg Ser	atc Ile	gag Glu 190	aac Asn	cgt Arg	576
gcg Ala	aga Arg	ttc Phe 195	act Thr	ttg Leu	gaa Glu	gtg Val	gtt Val 200	gat Asp	gcc Ala	gtt Val	gta Val	gat Asp 205	gca Ala	gtt Val	ggg Gly	624
gcc Ala	gaa Glu 210	aga Arg	acc Thr	tcc Ser	atc Ile	aga Arg 215	ttc Phe	tct Ser	cct Pro	tac Tyr	ggg Gly 220	act Thr	ttt Phe	ggg Gly	acc Thr	672
atg	tcc	ggg	ggg	gag	aac	cct	ggc	atc	gtt	gcc	caa	tat	gca	tac	gtc	720

Met 225	Ser	Gly	Gly	Glu	Asn 230	Pro	Gly	Ile	Val	Ala 235	Gln	Tyr	Ala	Tyr	Val 240	
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atc Ile	gat Asp	ttg Leu	gtc Val 260	gag Glu	cct Pro	cgt Arg	gtg Val	acc Thr 265	gac Asp	cca Pro	ttc Phe	cta Leu	cca Pro 270	gaa Glu	ttc Phe	816
gag Glu	aag Lys	tgg Trp 275	ttc Phe	aag Lys	gaa Glu	ggt Gly	acc Thr 280	aac Asn	gaa Glu	ttc Phe	atc Ile	tac Tyr 285	tct Ser	atc Ile	tgg Trp	864
aag Lys	ggt Gly 290	cca Pro	gtt Val	ctc Leu	aga Arg	gtt Val 295	ggt Gly	aac Asn	tat Tyr	gct Ala	ttg Leu 300	gac Asp	cca Pro	gat Asp	caa Gln	912
gct Ala 305	act Thr	atc Ile	gac Asp	tct Ser	aag Lys 310	aag Lys	cct Pro	aac Asn	acc Thr	ttg Leu 315	atc Ile	ggt Gly	tac Tyr	ggt Gly	aga Arg 320	960
tcc Ser	ttt Phe	att Ile	gcc Ala	aac Asn 325	cca Pro	gac Asp	ttg Leu	gtg Val	tac Tyr 330	cgt Arg	ttg Leu	gaa Glu	aag Lys	ggt Gly 335	ttg Leu	1008
cca Pro	ttg Leu	aac Asn	aag Lys 340	tat Tyr	gat Asp	aga Arg	aac Asn	acc Thr 345	ttc Phe	tac Tyr	acc Thr	ttc Phe	acc Thr 350	aaa Lys	gag Glu	1056
gg																1058

<210> 22
 <211> 352
 <212> PRT
 <213> Candida kefyr

<400> 22

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Ser	Gln	Tyr 35	Pro	Gly	Thr	Leu	Ile 40	Ile	Thr	Glu	Gly	Thr 45	Phe	Pro	Ser
Ala 50	Gln	Ser	Gly	Gly	Tyr	Pro 55	Asn	Val	Pro	Gly	Ile 60	Trp	Ser	Lys	Glu
Gln 65	Leu	Ala	Glu	Trp	Lys 70	Lys	Ile	Phe	Asn 75	Ala	Ile	His	Glu	Asn 80	Lys
Ser	Phe	Val	Trp	Val 85	Gln	Leu	Trp	Val	Leu 90	Gly	Arg	Gln	Ala	Trp 95	Pro

Glu Val Leu Lys Lys Glu Gly Leu Arg Tyr Asp Ser Ala Thr Asp Asp
 100 105 110
 Leu Tyr Met Gly Glu Glu Glu Lys Glu Arg Ala Leu Lys Ala Asn Asn
 115 120 125
 Pro Gln His Gly Ile Thr Lys Glu Glu Ile Lys Gln Tyr Ile Lys Glu
 130 135 140
 Tyr Val Asp Ala Ala Lys Lys Ala Ile Asp Ala Gly Ala Asp Gly Val
 145 150 155 160
 Gln Ile His Ser Ala Asn Gly Tyr Leu Leu Asn Gln Phe Leu Asp Pro
 165 170 175
 Ile Ser Asn Asn Arg Thr Asp Glu Tyr Gly Gly Ser Ile Glu Asn Arg
 180 185 190
 Ala Arg Phe Thr Leu Glu Val Val Asp Ala Val Val Asp Ala Val Gly
 195 200 205
 Ala Glu Arg Thr Ser Ile Arg Phe Ser Pro Tyr Gly Thr Phe Gly Thr
 210 215 220
 Met Ser Gly Gly Glu Asn Pro Gly Ile Val Ala Gln Tyr Ala Tyr Val
 225 230 235 240
 Ile Gly Glu Leu Glu Lys Arg Ala Arg Ala Gly Lys Arg Leu Ala Phe
 245 250 255
 Ile Asp Leu Val Glu Pro Arg Val Thr Asp Pro Phe Leu Pro Glu Phe
 260 265 270
 Glu Lys Trp Phe Lys Glu Gly Thr Asn Glu Phe Ile Tyr Ser Ile Trp
 275 280 285
 Lys Gly Pro Val Leu Arg Val Gly Asn Tyr Ala Leu Asp Pro Asp Gln
 290 295 300
 Ala Thr Ile Asp Ser Lys Lys Pro Asn Thr Leu Ile Gly Tyr Gly Arg
 305 310 315 320
 Ser Phe Ile Ala Asn Pro Asp Leu Val Tyr Arg Leu Glu Lys Gly Leu
 325 330 335
 Pro Leu Asn Lys Tyr Asp Arg Asn Thr Phe Tyr Thr Phe Thr Lys Glu
 340 345 350

<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> IA1 (antisense primer for upstream region)

<400> 23
attcctcggc ccattcagtg ttggg 25

<210> 24
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> IS1 (sense primer for downstream region)

<400> 24
ggtgtaccgt ttggaaaagg gtttgc 26

<210> 25
<211> 1796
<212> DNA
<213> Candida kefyr

<400> 25
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aattaattat atcaacaaac tgtcgagatg tcgtacatga actttgaccc taagccattg 180
ggagacacca atatcttcaa gccaatcaag atcggttaaca atgagctaaa acacagagta 240
gtcatgccag cattgactag aatgagagcc attgcaccag gaaacatccc aaacactgaa 300
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gagtacgtgg atgctgcaa gaaagccatc gatgcagggtg cagacgggtg gcaaattccat 720
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gccaaaccag acttggtgta ccgtttggaa aagggtttgc cattgaacaa gtatgataga	1260
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ctaccattaa gtctttgtgc gaaatgagta accttatatt aataaaagat gtgaagtgtg	1740
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<210> 26
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense Primer

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<210> 27
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense Primer

<400> 27	
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<210> 28
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 <213> Candida kefyr

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ggtaacaatg agctaaaaca cagagtagtc atgccagcat tgactagaat gagagccatt	180
gcaccaggaa acatcccaaa cactgaatgg gccgaggaat actacagaca acgttctcaa	240
taccctggta cccttattat cacggaaggt actttccctt ctgcgcaatc aggtgggttac	300

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gctcaatatg	catacgtcat	tggtgagttg	gaaaagagag	ctagagctgg	caagagattg	900
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<210> 29
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 <213> Artificial Sequence

<220>
 <223> Primer ExS

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<210> 30
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<220>
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<210> 31
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<220>

<223> degenerate Primer encoding SEQ ID NO:19

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<222> (3)..(3)

<223> I

<220>

<221> modified_base

<222> (9)..(9)

<223> I

<220>

<221> misc_feature

<222> (24)..(24)

<223> n is a or c or g or t

<400> 31

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26